The Tenth U.S.-Korea Forum on Nanotechnology, Boston

Wafer Level Fabrication of Nanowell Array Biochip and Its Characterization

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October 15, 2013

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Acknowledgements

❖ Funding from KRF Center for Human Sensing Systems (ERC) at Hanyang University.

❖ Funding from BK21 Plus Program for Bio Nano Technology at Hanyang University.

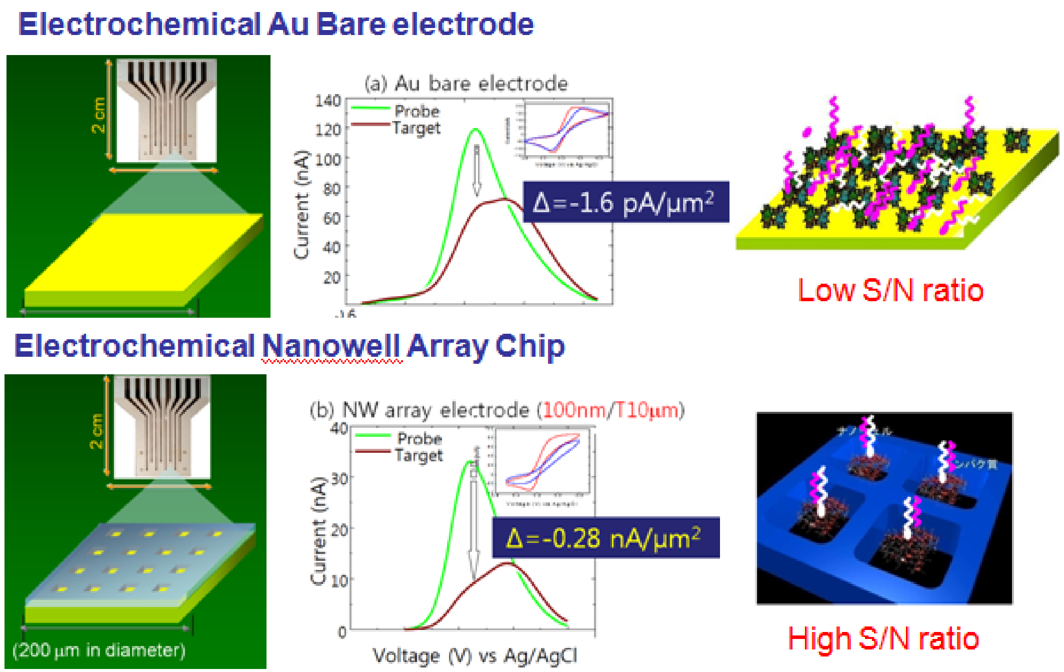
❖ Prof. H. Y. Lee of Northeastern University for biochip design and characterization

Seongpil Hwang et,al., Chemworld (2010) 05, 25--28

Contents

Why nanowell array (NWA) biochip?

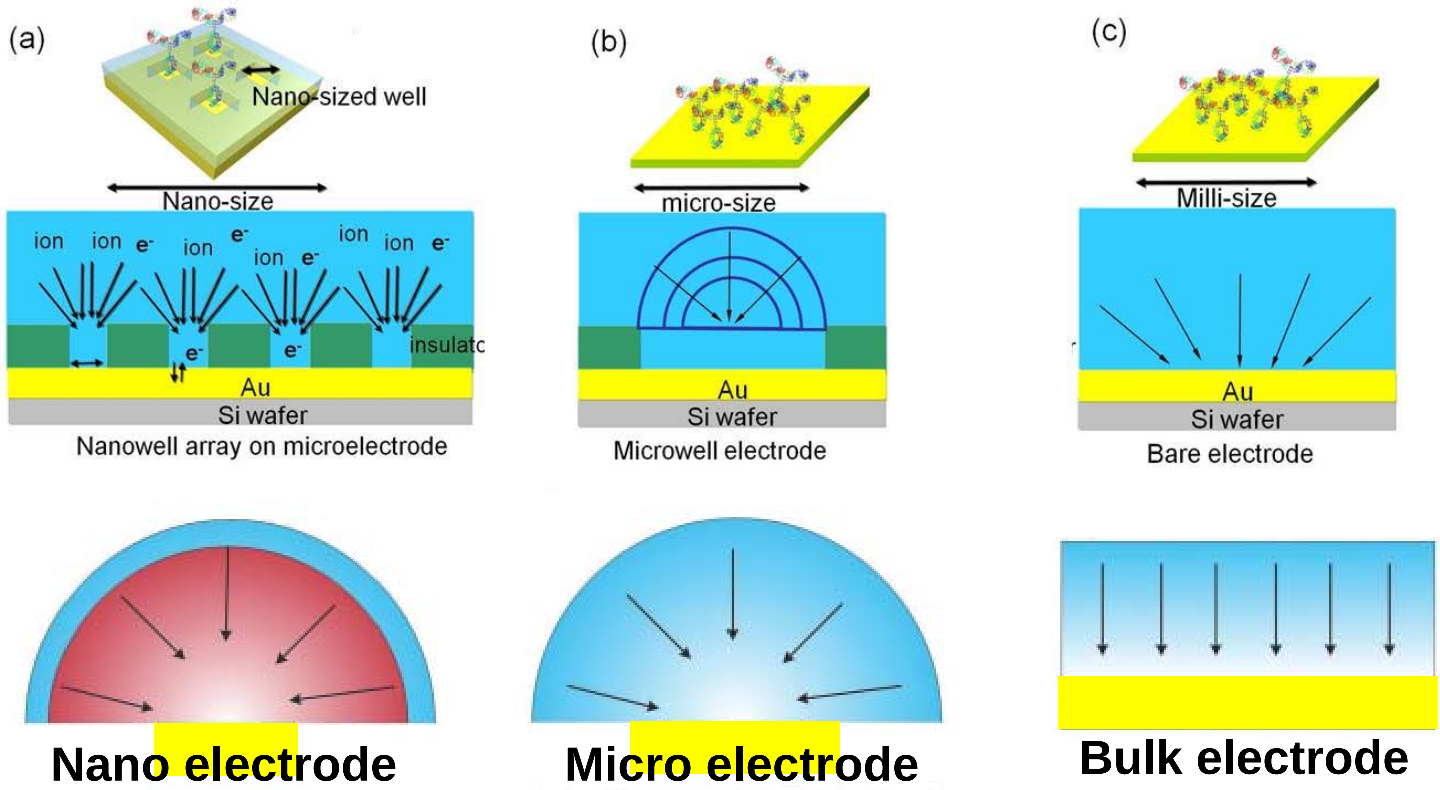
- Rapid response time (small RC delay)
- Small iR drop (reduction of noise and Increased sensitivity)
- High mass-transport rate leading to steady-state diffusion (high S/N),
  Enhanced faradic currents (radial diffusion)
- Highly sensitive and specific biomolecular assay with a small amount



[Ref.] H.Y.Lee,T.Kawai et, al., Langmuir 21, p.6025-6029 (2005).

[Ref.] D. Wei et. al., Lab Chip, 9 (2009) pp. 2123-2131

Phenomenon at Nanowell Electrode

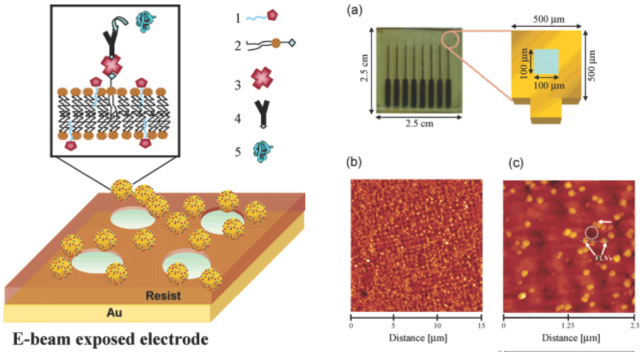


- Higher mass transfer rates in micro/nanoelectrode due to **radial diffusion**
- Bulk electrode that operate via **planar diffusion**

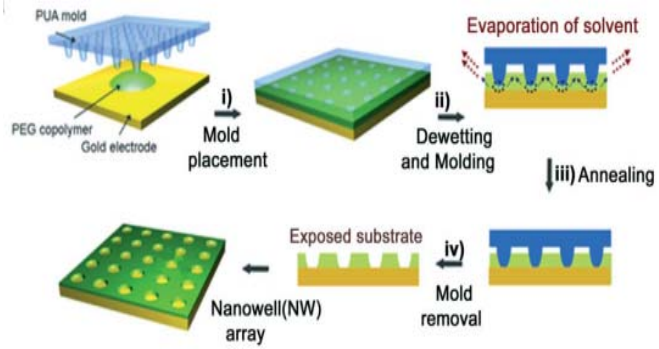
[Ref.] J. C. Hulteen et .al., J. Chem. Soc., Faraday Trans. 92 (1996) 4029
T. J. Davies and R. G. Compton, J. Electroanal. Chem. 585 (2005) 63
S. Hwang et,al., Chemworld (2010) 05, 25--28

Reported fabrication methods of NWA biochip

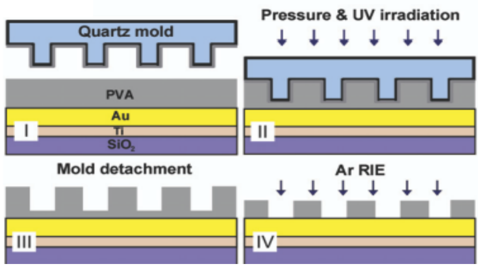
- **Jung et al. Langmuir 21, 6025-6029 (2005)**
  - Method : **E-beam lithography**
  - Limitation : Small area, Low throughput



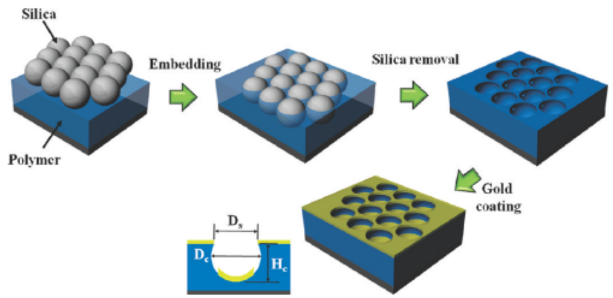
- **Kim P. et al. Adv. Mater. 20, 31-36 (2008)**
  - Method : **Soft lithography with PUA mold**
  - Limitation : Small area, Low reliability



- **Lee et al. Lab Chip 9, 132-139 (2009)**
  - Method : **UV nanoimprinting (UV-NIL)**
  - Limitation : Costly mold, Low repeatability

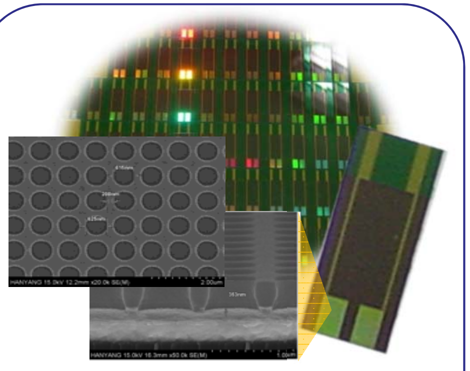


- **Lee et al. Anal. Chem. 83, 9174-9180 (2011)**
  - Method : **Colloidal lithography (CL)**
  - Limitation : Non-uniform nanoarray structure

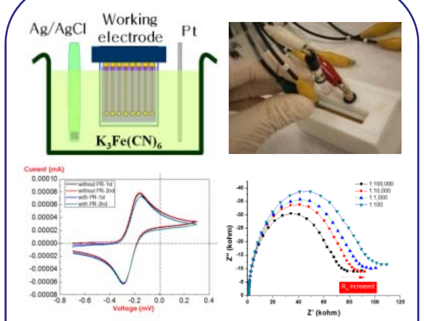


Research Motivation

- **Fabrication of highly sensitive biochip based on nanowell array (NWA)**
- **Development of manufacturing process for mass production**
- **Characterization of NWA biochip using electrochemical analysis**



Nano well array device



Electrochemical Sensing System

Nano Array Biochips

By geometries of electrodes

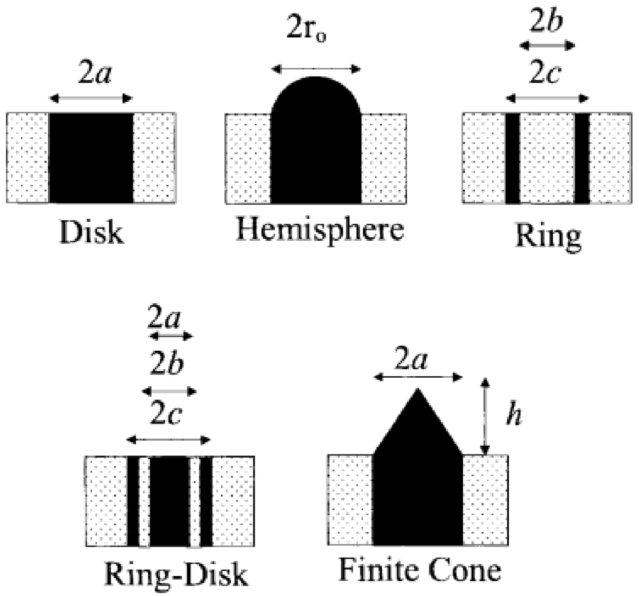
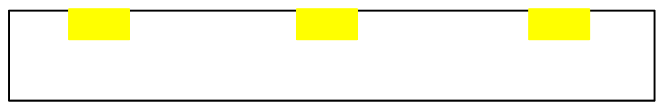


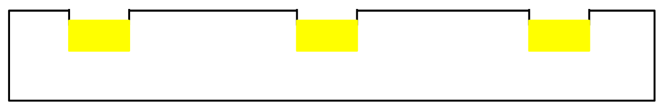
Fig. 1. Popular UME geometries. *a*: radius of disk or finite conical electrode; r_0: radius of hemispherical electrode; *b*: inner radius of ring electrode; *c*: outer radius of ring electrode.

By position of electrodes

(a) Inlaid electrodes



(b) Recessed electrodes

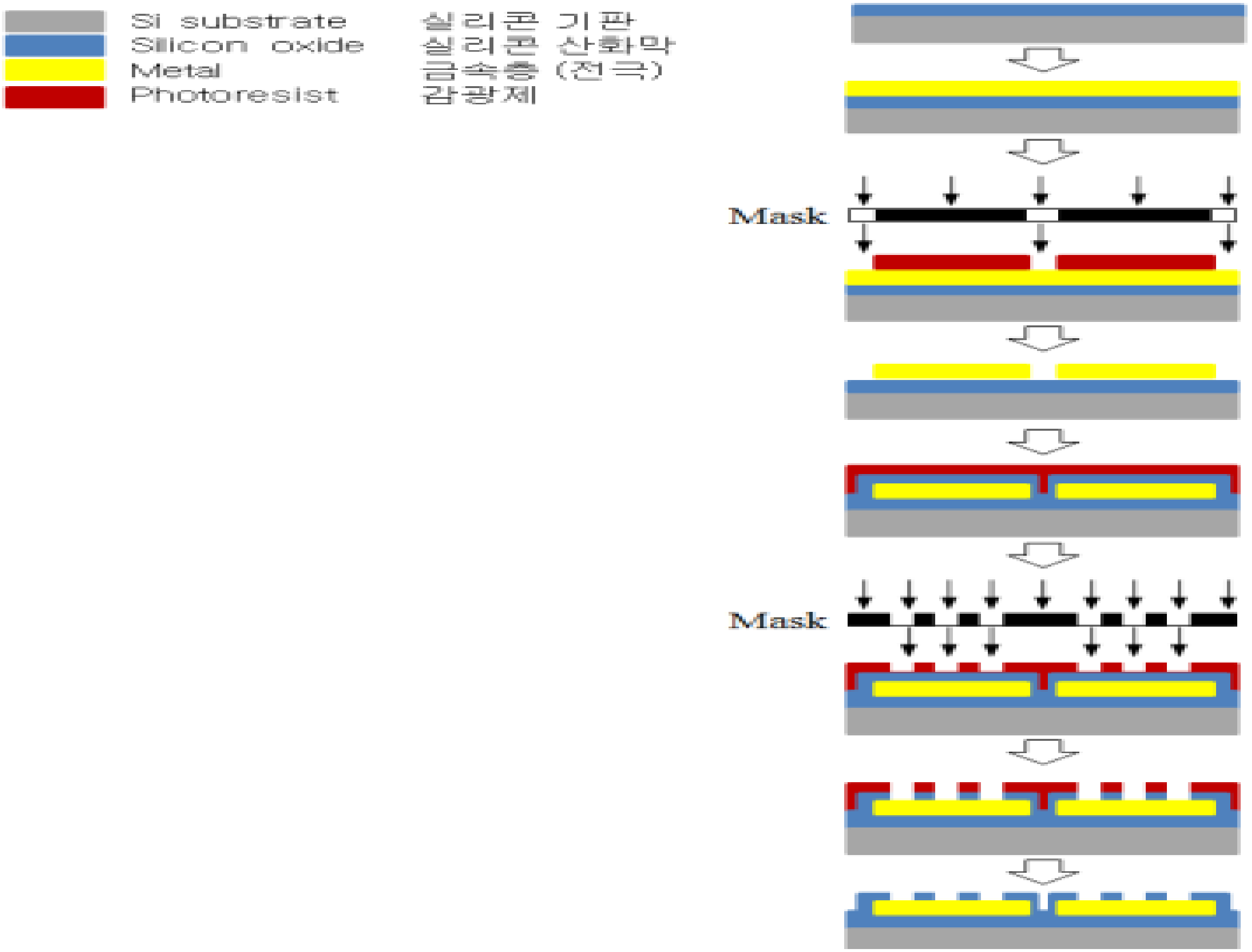


In this study, nanowell array (NWA) → Nanoelectrode arrays (NEAs) and recessed type

[Ref.] C. G. Zoski, Electroanalysis, 14 (2002) pp. 1041-1051

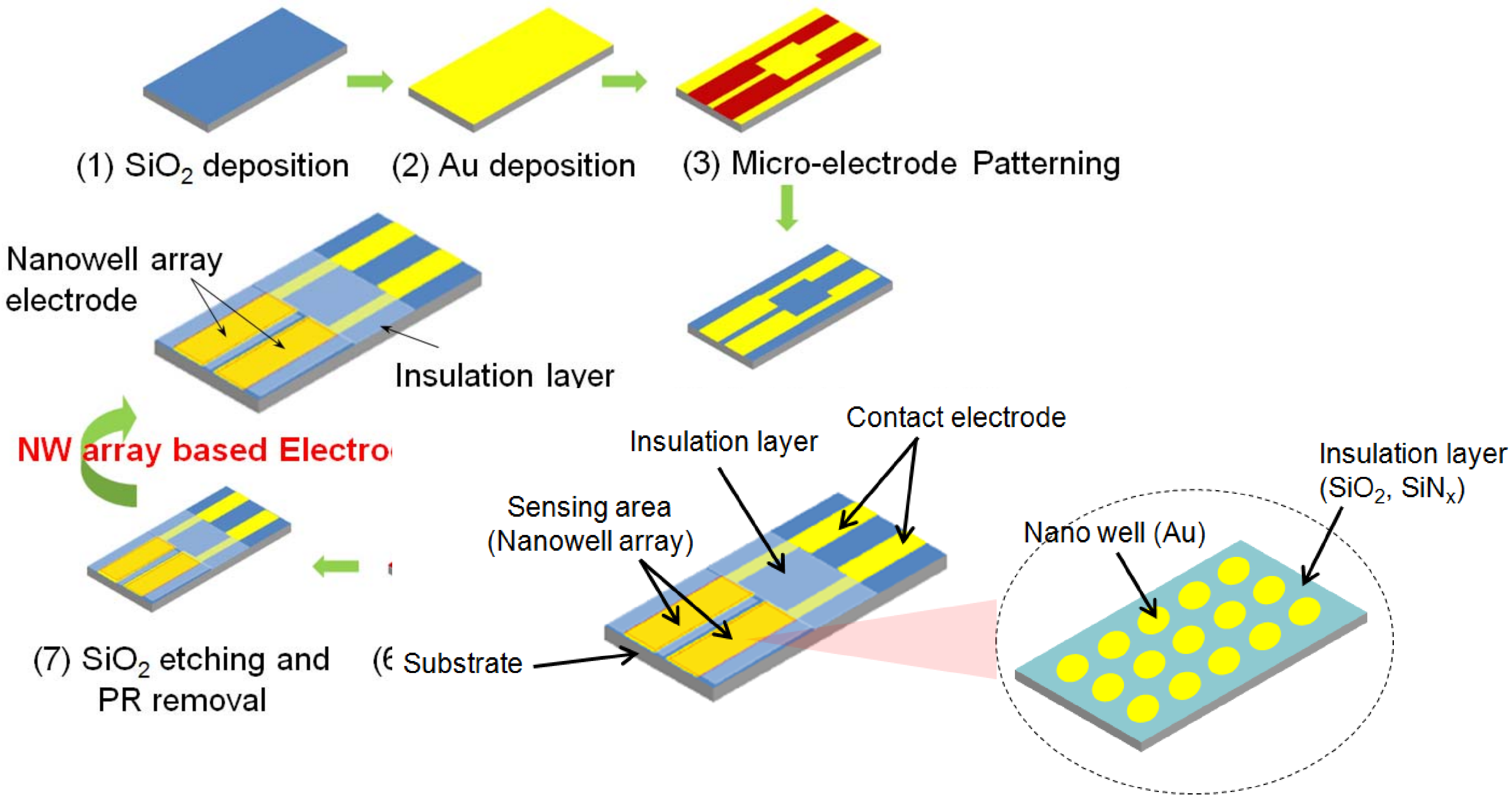
Fabrication Process of NWA Biochip

Process Procedure (Vertical view)



[Ref.] M. S. Cha et. al., J. Nanosci. Nanotechnol. 13 (2013) pp. 5245 -5249
J. K. Lee et. al., J. Biotechnology (2013) In Press

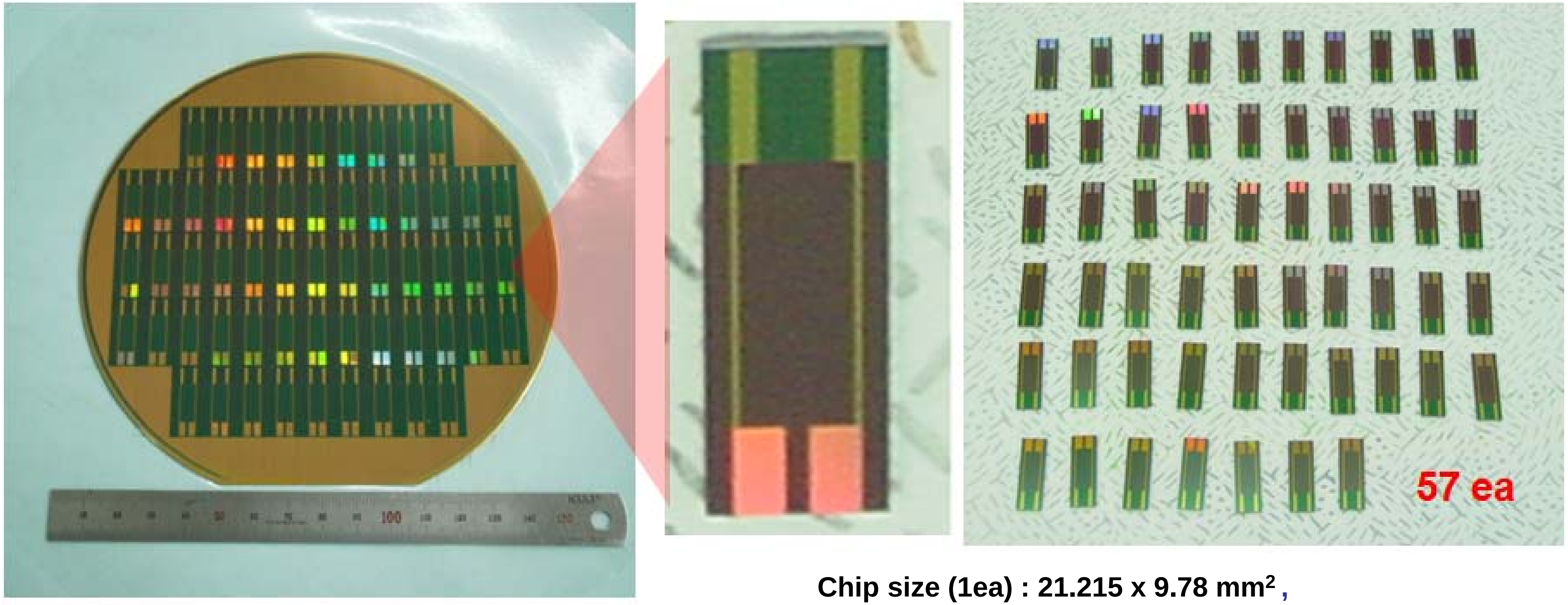
Fabrication Process of NWA Biochip



Nanowell Array Biosensor

Fabricated NWA Biochip

- **Mass production of NWA chips**

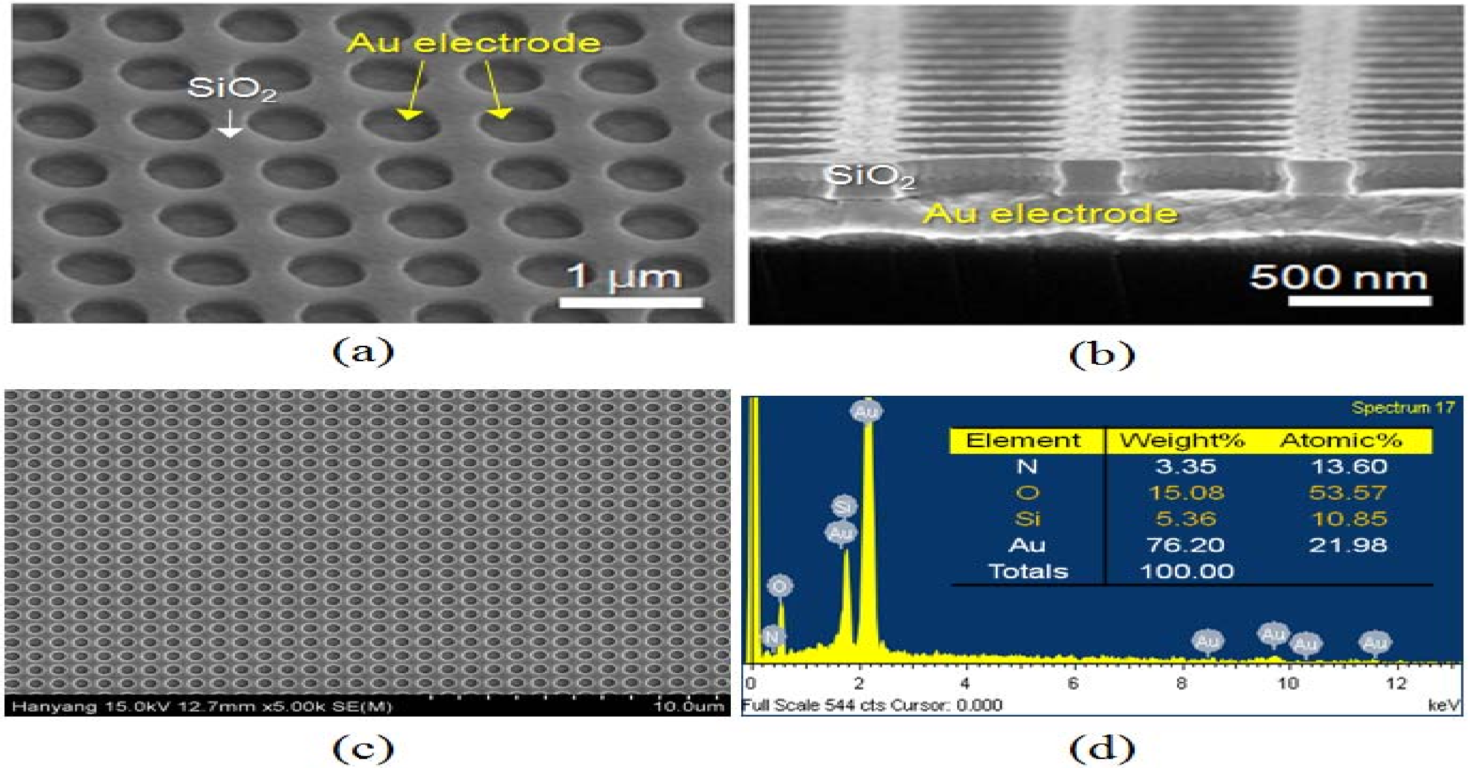


Chip size (1ea) : 21.215 x 9.78 mm^2 ,
Electrode area = 4 x 2 mm^2

(a) (b) (c)

Fabricated NWA Biochip

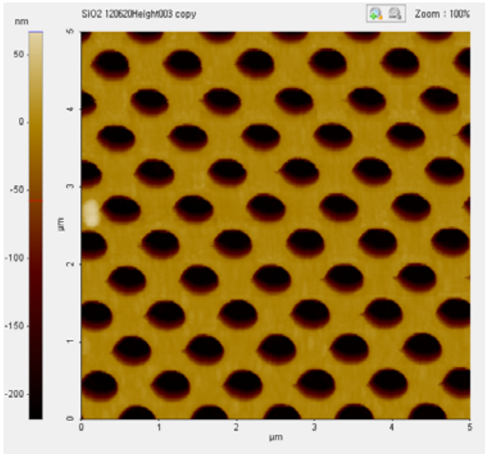
- **FE-SEM & EDS result**

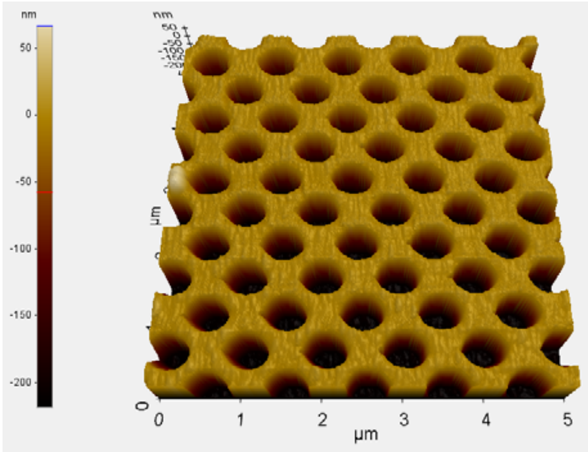


(a, b, c) FE-SEM image of nanowell array structure
(d) EDS result

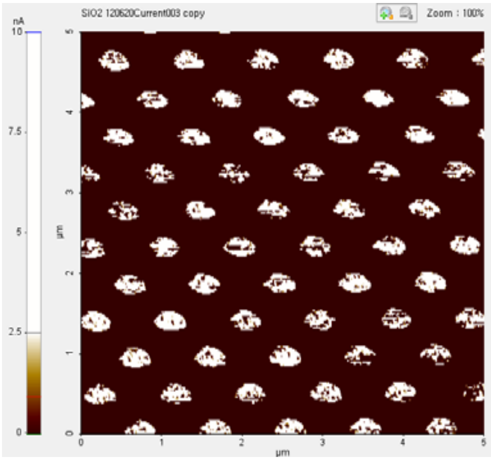
I-AFM Result of SiO_2 NWA chip

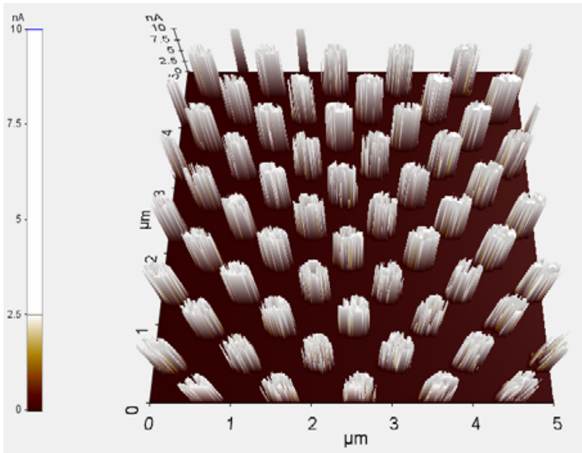
❖ Topography (5 μm²)

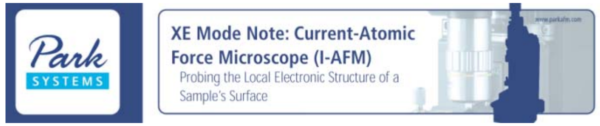




❖ Current AFM image (5 μm²)







❖ AFM conditions

Head Mode	I-AFM
Source	Current
Data Width	512 (pxl)
Date Height	256 (pxl)
X Scan Size	5 (μm)
Y Scan Size	5 (μm)
Scan Rate	0.5 (Hz)
Z Servo Gain	17
Set Point	4.02 (nN)
Sample Bias	0.1 (V)

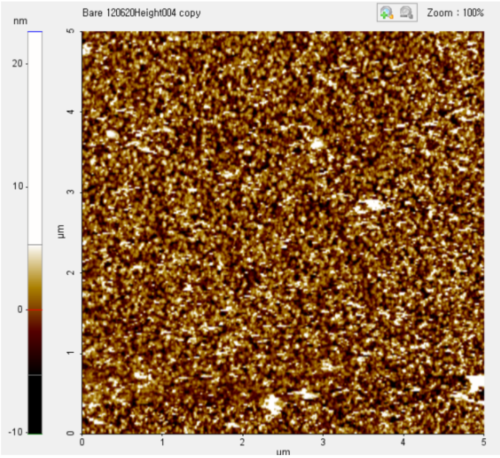
❖ AFM Tip Information

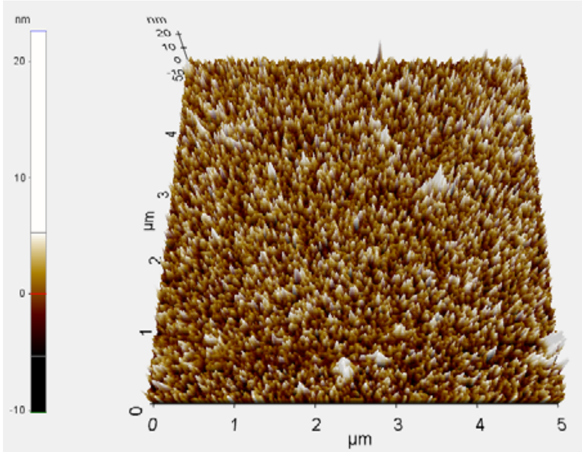
Model Name : PPP-CONTSCPT
Manufacturer : Nanosensors

Point Probe Plus Contact Mode Short Cantilever PtIr5 Coating

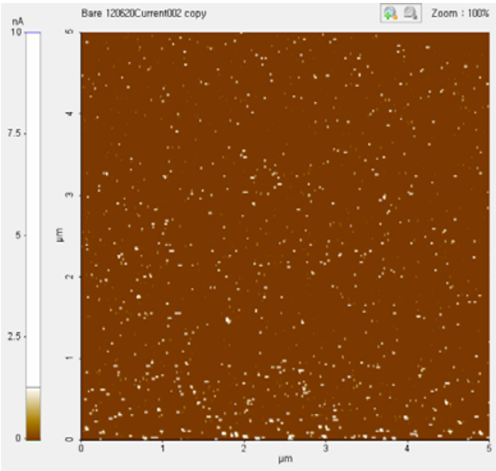
I-AFM Result of Bare Au chip

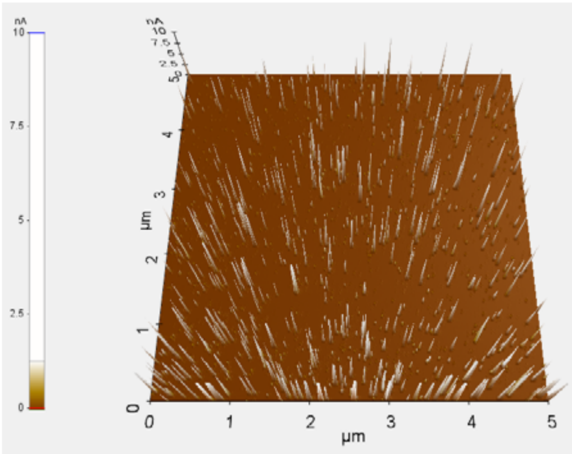
❖ Topography (5 μm^2)

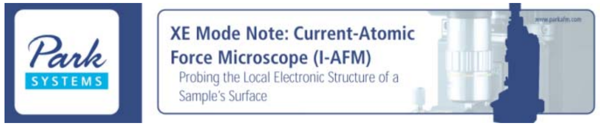




❖ Current AFM image (5 μm^2)







❖ AFM conditions

Head Mode	I-AFM
Source	Current
Data Width	512 (pxl)
Date Height	256 (pxl)
X Scan Size	5 (μm)
Y Scan Size	5 (μm)
Scan Rate	0.8 (Hz)
Z Servo Gain	1
Set Point	5 (nN)
Sample Bias	0.1 (V)

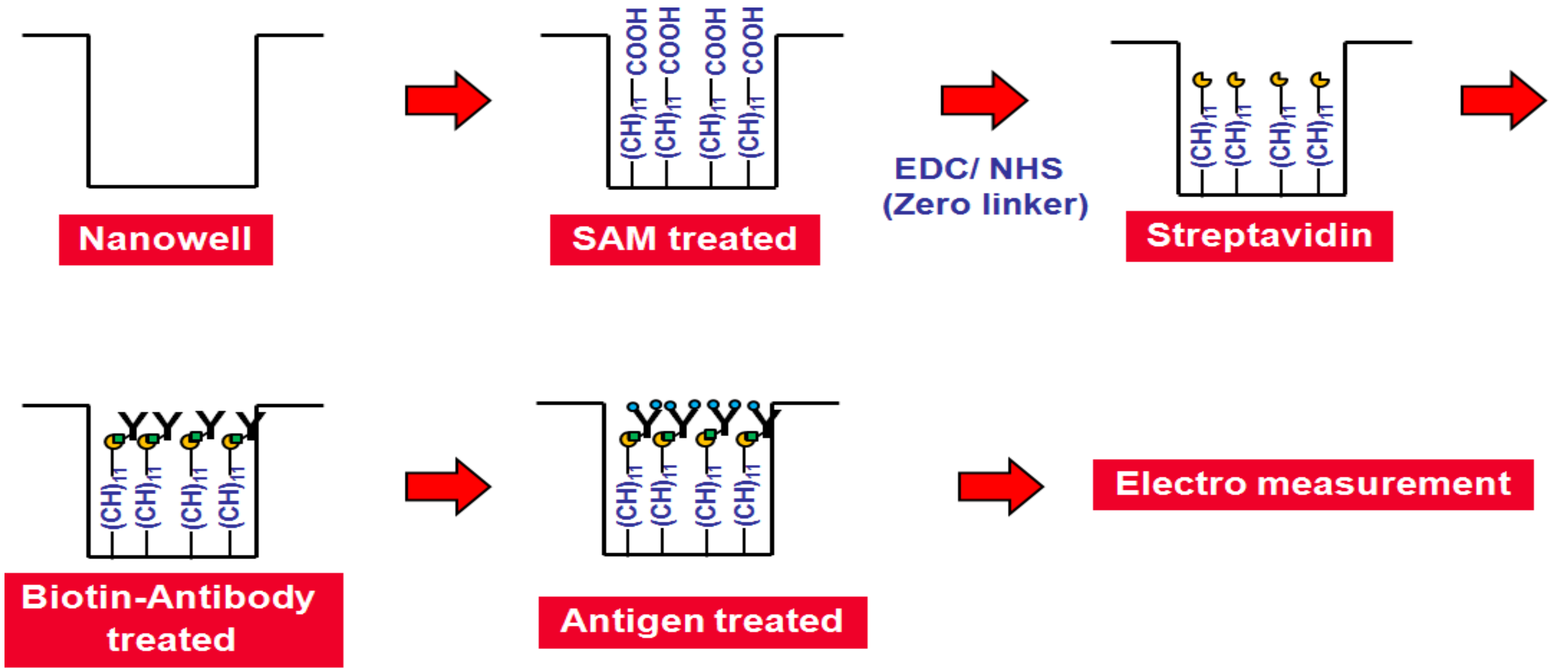
❖ AFM Tip Information

Model Name : PPP-CONTSCPT
Manufacturer : Nanosensors

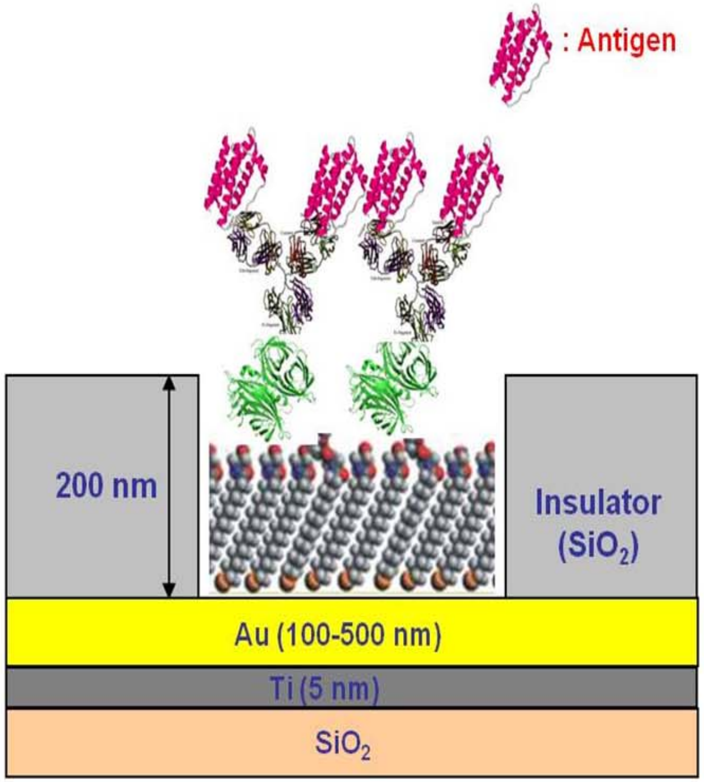
Point Probe Plus Contact Mode Short Cantilever PtIr5 Coating

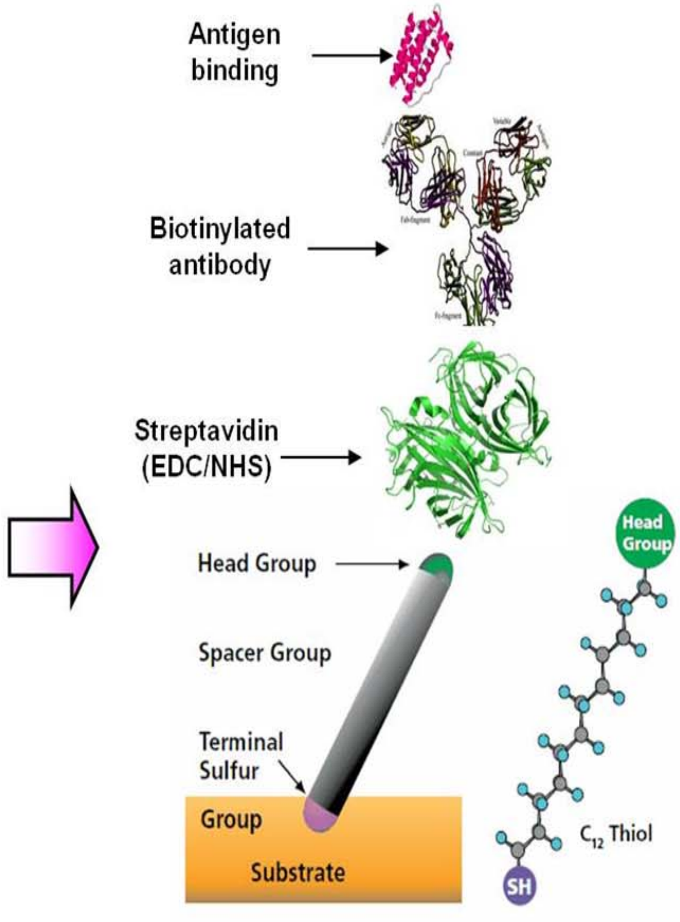
Preparation of Immuno-affinity layer

- **Self-Assembled Monolayer (SAM) on a nanowell(NW) electrode**
  : incubating 10 mM, 11-mercaptoundecanoic acid (11-MUA) in anhydrous ethanol for 1 hour at room temperature (linker)
- **The formation of active ester functional group**
  : 50 mM EDC and 50 mM NHS in pH 5.5 sodium acetate buffer treatment
- **Immobilization**
  : Streptavidin 1 mg/ml in PBS for 30 min at room temperature, 10 μg/ml biotinylated antibody immobilized on electrode
- **Antigen (Target material)**
  : Stress-induced-phosphoprotein-1 (STIP-1), Biomarker of Ovarian Cancer



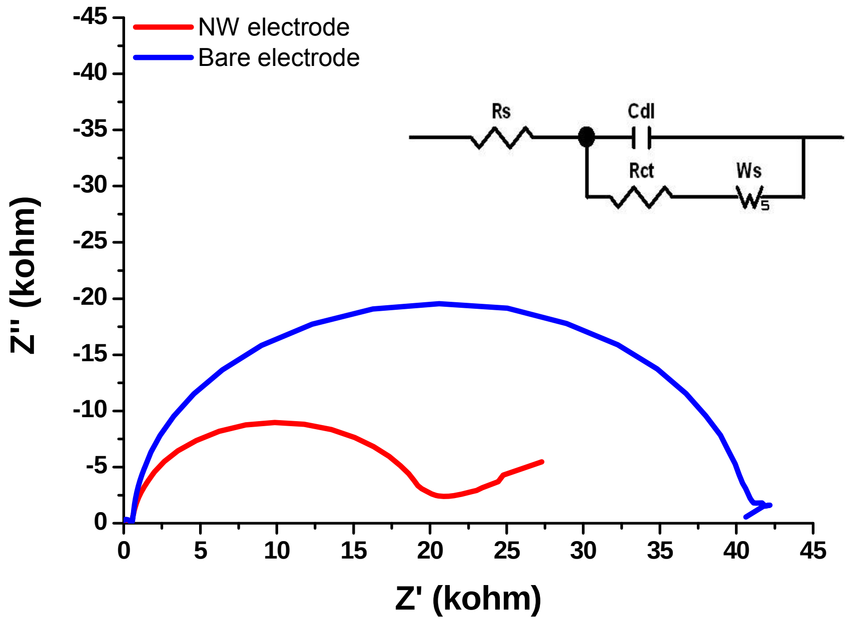
Schematic of Immuno-affinity layer



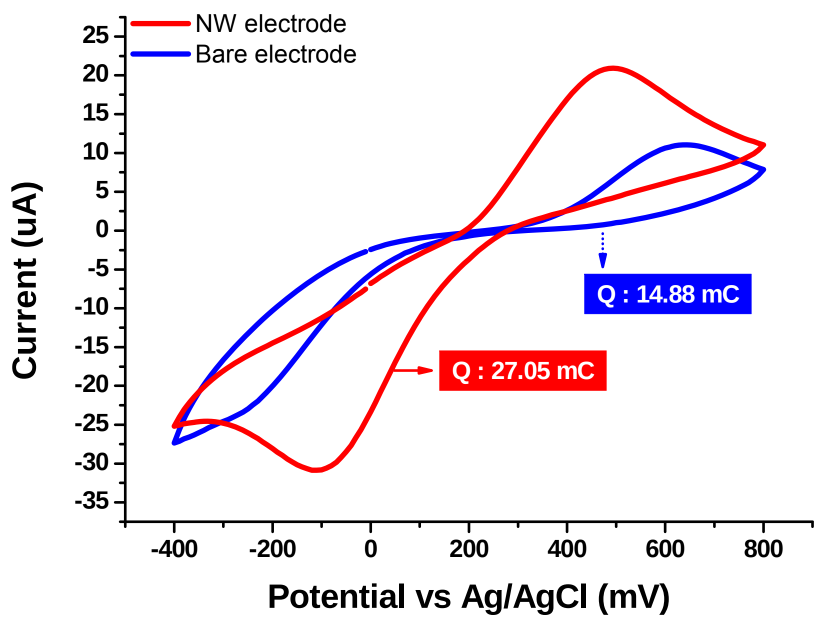


Electrochemical characterization (1)

❖ **Electron transfer imaging using EIS and CV of Nanowell and Bare electrode**



(a) Nyquist plot from EIS



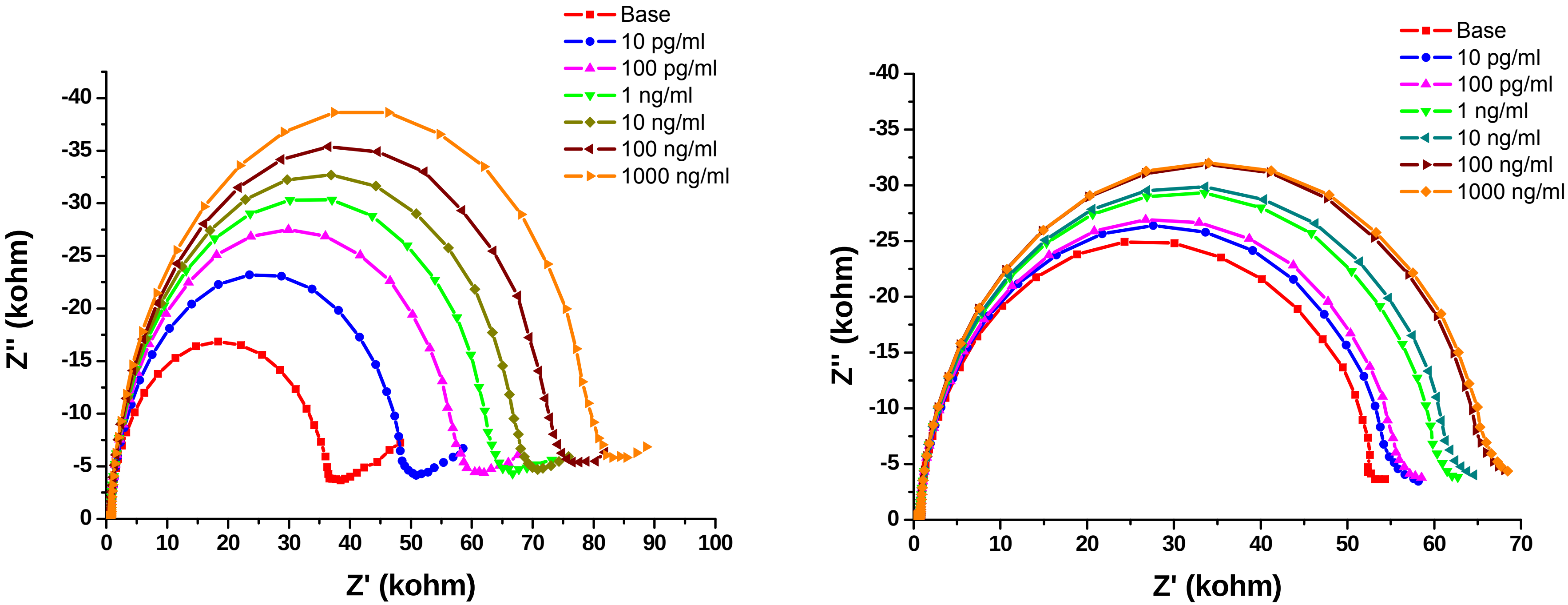
(b) Cyclic voltammogram

- **The Rct of NW electrode is 20.87 kohm and bare electrode is 42.98 kohm.**
- **NW electrode has higher current and lower charge transfer resistance than bare.**
- **The electrons in NW's double layer easily pass from electrode to solution than bare.**

[Ref.] J. K. Lee et. al., J. Biotechnology (2013) In Press

Electrochemical characterization (2)

❖ **Impedance measurement of Nanowell and Bare electrode**

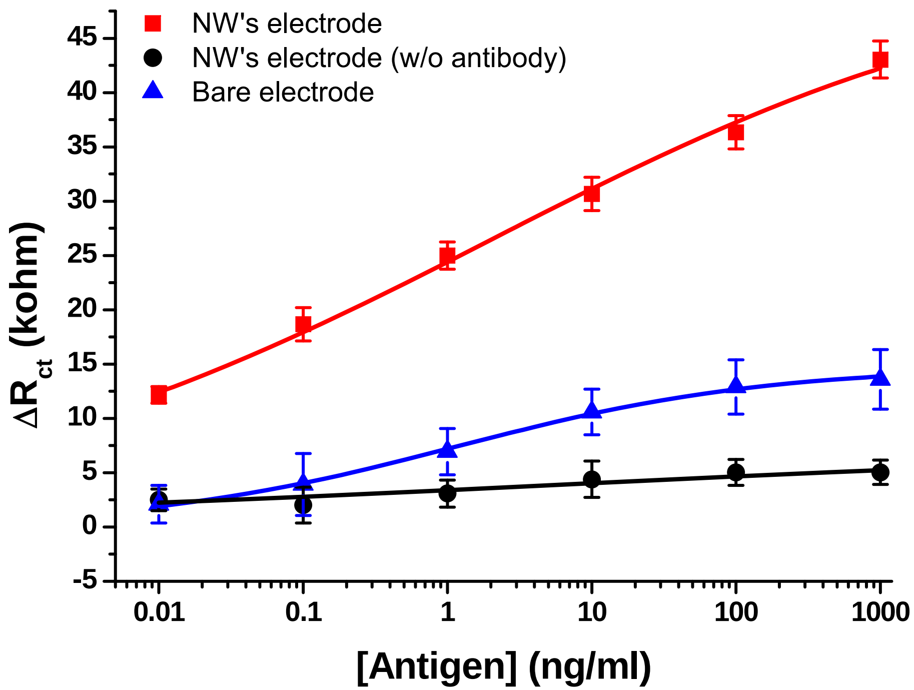


(a) Nanowell electrode **(b) Bare electrode**

- **After treatment of streptavidin and biotinlylated antibody (base), then we treated antigen changing with its concentration. (10 pg/ml ~ 1000 ng/ml)**
- **The base signal of bare electrode is 52 kohm that is 43 % increased compare with the base signal (37 kohm) of NW electrode.**
- **The Rct of NW electrode with the increase of antigen increased very evidently.**
- **The differences at bare electrode are smaller than differences at NW electrode.**

Electrochemical characterization (3)

❖ **Limit of detection (LOD) estimation from standard curve**



- **The movement of Rct by changing antigen concentration was showed.**
- **The signal at low concentration in bare electrode interferes. This indicates low SNR because of non-specific binding.**
- **At NW electrode, LOD was estimated to be approximately 10 pg/ml, which is more than 100-fold improved LOD in comparion to the bare electrode.**
- **NW electrode has high sensitivity, selectivity and very low LOD.**

[Ref.] J. K. Lee et. al., J. Biotechnology (2013) In Press

Main issues for a chip design

- **How can we design the patterns' dimensions?**

The ideal case is to have the electrodes at an intermediate density to maintain radial diffusion. Many researchers have used this following equation,

$R_0 > 6R_b$, H_{PR} (The ideal case)

R_0 : One half inter-well distance, R_b : well radius, H_{PR} : well height

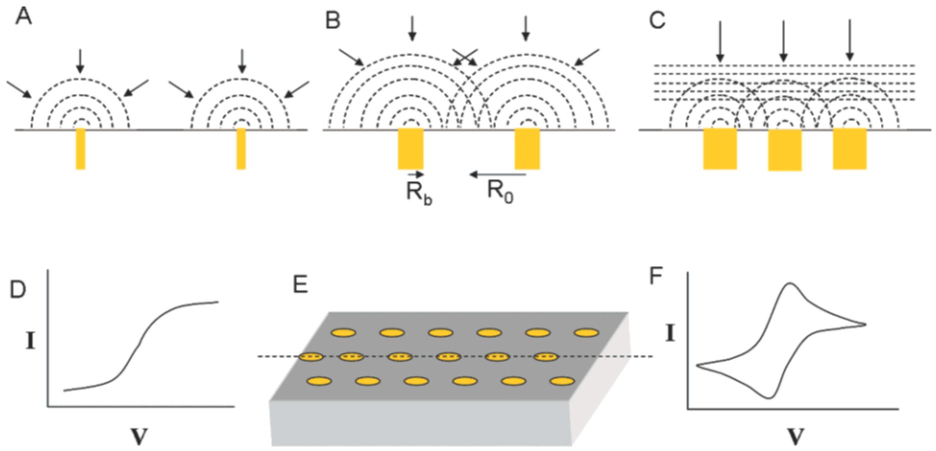


Figure 1. Schematic of electrode ensembles of different size and density showing (A) radial diffusion, (B) overlapping radial diffusion, and (C) planar diffusion. (D and F) Cyclic voltammograms for the diffusion scenario in A and C, respectively. (E) Electrode ensemble with metal electrodes represented by yellow circles surrounded by gray insulating material. Microelectrode radius, R_b, and diffusion zone radius, R_0, are shown.

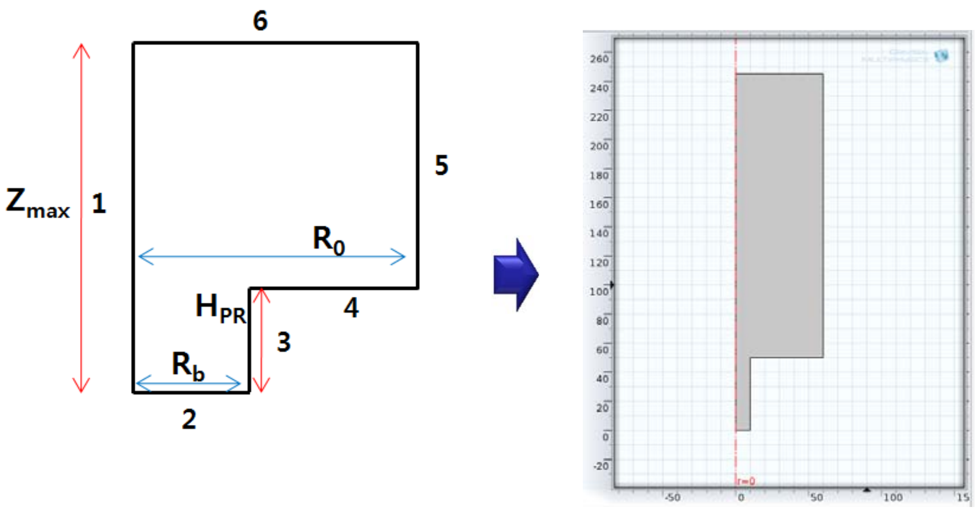
[Ref.] C. N. LaFratta and D. R. Walt, Chemical Reviews, 108 (2008) pp. 614-637
C. G. Zoski and M. Wijesinghe, Israel Journal of Chemistry, 50 (2010) pp. 347 -359

Modeling for Simulation

Table 1. Simulation geometries of various cases of nanowell array chip

Case	R_b (μm)	R_0 (μm)	Spacing (μm)	C To C (μm)	Hpr (μm)	Zmax (μm)	Scan rate (v/s)
1	0.25	0.5	0.5	1	0.2	245	0.1
2	0.25	1.5	2.5	3	0.2		
3	0.25	2.5	4.5	5	0.2		
4	0.25	0.5	0.5	1	0.5		
5	0.25	1.5	2.5	3	0.5		
6	0.25	2.5	4.5	5	0.5		

*Cell height, $Z_{max} = \sqrt{6Dt_{max}}$, t_{max} : the time for ½ CV scan, D : diffusion coefficient



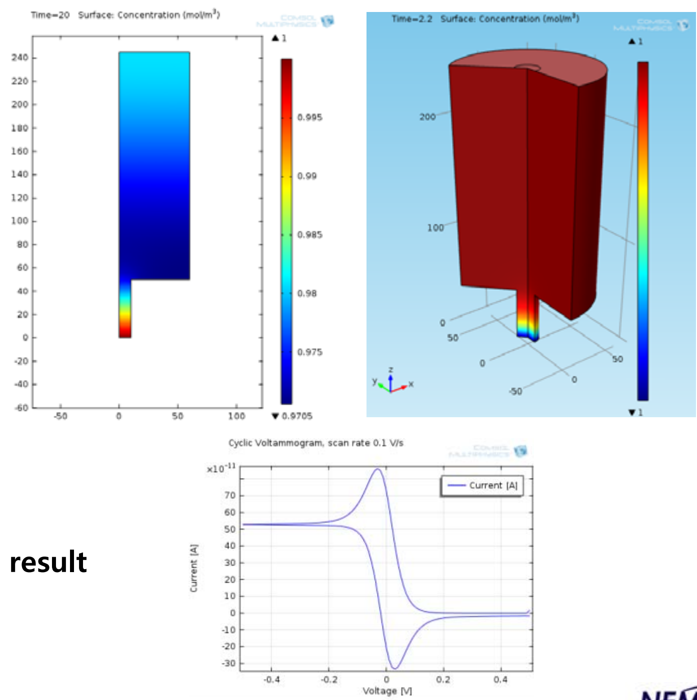


Fig. 2. 2D model of diffusion domain of nanowell and examples of simulation result

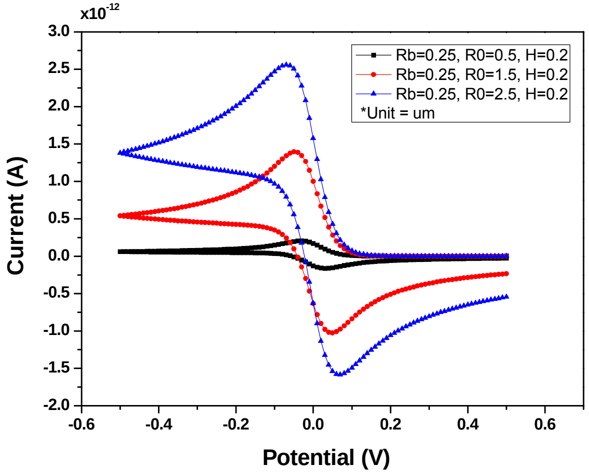
COMSOL Multiphysics® Modeling (Ver. 4.3)

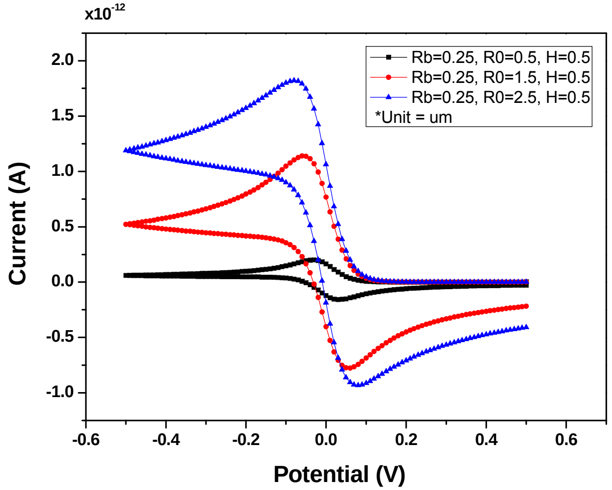
Simulation Results

- **Cyclic voltammograms as a function of different R_0 (1/2 Interwell spacing)**

Case	Rb (µm)	R0 (µm)	Spacing (µm)	C To C (µm)	Hpr (µm)	Zmax (h)	Scan rate (v/s)
1	0.25	0.5	0.5	1	0.2	245	0.1
2	0.25	1.5	2.5	3	0.2	245	0.1
3	0.25	2.5	4.5	5	0.2	245	0.1

Case	Rb (µm)	R0 (µm)	Spacing (µm)	C To C (µm)	Hpr (µm)	Zmax (h)	Scan rate (v/s)
4	0.25	0.5	0.5	1	0.5	245	0.1
5	0.25	1.5	2.5	3	0.5	245	0.1
6	0.25	2.5	4.5	5	0.5	245	0.1





- **The NWA chip becomes more effective when R_0 value is larger and the height of nanowell is smaller.**
- **The simulation study will be continuously performed for the more understanding of nanowell array chip.**

Summary

- A highly sensitive biochip was developed by using nanowell array electrode structure.

- The fabrication process was developed for mass production of nanowell array biochip.

- Materials and design optimization needed for future study

Seongpil Hwang et,al., Chemworld (2010) 05, 25--28

경청해주셔서 감사합니다.

Thank You for Your Attention.

HANYANG UNIVERSITY